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Ringer's ethyl pyruvate solution ameliorates ischemia/reperfusion-induced intestinal mucosal injury in rats

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Objective: Pyruvate has been shown to be protective in numerous *in vitro* and *in vivo* models of oxidant-mediated cellular or organ system injury. Unfortunately, the usefulness of pyruvate as a therapeutic agent is abrogated by its very poor stability in solution. In an effort to take advantage of the ability of pyruvate to scavenge reactive oxygen species while avoiding the problems associated with the instability of pyruvate in solution, we sought to determine whether a simple derivative, ethyl pyruvate, would be protective in an animal model of reactive oxygen species-mediated tissue injury, namely mesenteric ischemia and reperfusion in rats.

Design: Prospective, randomized trial.

Setting: Animal research center.

Subjects: Male Sprague-Dawley rats.

Interventions: Under general anesthesia, rats were subjected to 60 mins of mesenteric ischemia followed by 60 mins of reperfusion. Controls (n = 6) received intravenous lactated Ringer's solution according this dosing schedule: 1.5 mL/kg bolus before ischemia, 3.0 mL/kg bolus before resuscitation, and 1.5 $mL \cdot kg^{-1} \cdot hr^{-1}$ by continuous infusion. Two experimental groups received similar volumes of either pyruvate (n = 6 each) or ethyl pyruvate (n = 9) solution made up exactly like lactated Ringer's solution except for the substitution of either pyruvate or ethyl pyruvate for lactate, respectively.

Measurements and Main Results: To obtain tissues for assessing mucosal permeability and histology, five 10-cm long segments of small intestine were obtained at the following time points: baseline, after 30 and 60 mins of ischemia, and after 30 and 60 mins of reperfusion. Mucosal permeability to fluorescein isothiocyanate dextran (molecular weight 4000 Da) was assessed *ex vivo* by using an everted gut sac method. Compared with controls, treatment of rats with either pyruvate solution or ethyl pyruvate solution significantly ameliorated the development of intestinal mucosal hyperpermeability during the reperfusion. Treatment with ethyl pyruvate solution also significantly decreased the extent of histologic mucosal damage after mesenteric reperfusion.

Conclusions: Treatment with Ringer's ethyl pyruvate solution ameliorated structural and functional damage to the intestinal mucosa in a rat model of mesenteric ischemia/reperfusion. Ethyl pyruvate solution warrants further evaluation as a novel therapeutic agent for preventing oxidant-mediated injury in various disease states. (Crit Care Med 2001; 29:1513–1518)

KEY WORDS: permeability, intestinal; oxidant stress; pyruvate; Ringer's lactate solution; resuscitation

Reactive oxygen species (ROS) have been implicated in the pathogenesis of the structural and functional alterations to tissues that are associated with a variety of pathologic processes, including sepsis and septic shock (1–3), thermal injury (4), doxirubicin-induced cardiomyopathy (5), hemorrhagic shock (6), and mesenteric ischemia/reperfusion (I/R) injury (7, 8). Potential sources of ROS in these and other conditions include the reaction catalyzed in neutrophils by reduced nicotinamide adenine dinucleotide phosphate oxidase, the reaction catalyzed in numerous cell types and in the extracellular milieu by the enzyme xanthine oxidase, and leakage of electrons from the electron transport chain in mitochondria. For the most part, the partially reduced form of oxygen that is produced by all of these sources is the radical species, superoxide anion ($O_2^{-\cdot}$), which is intrinsically only moderately reactive and injurious to cellular constituents. $O_2^{-\cdot}$, however, is converted to a much more reactive species, hydrogen peroxide (H_2O_2), by the enzyme superoxide dismutase. Both $O_2^{-\cdot}$ (9) and H_2O_2 (10) can react with another free radical, $NO^{\cdot}$, to form the highly toxic ROS, peroxynitrite ($ONOO^-$). Moreover, in a series of reactions catalyzed by iron (or certain other transition metal ions), $O_2^{-\cdot}$ and H_2O_2 are capable interacting to form to an extremely reactive moiety, hydroxyl radical ($OH^{\cdot}$) (11).

Pyruvate (CH_3COCOO^-), a small molecule that normally is regarded as a key intermediate in the oxidative or anaerobic metabolism of glucose, is also a potent and effective ROS scavenger. Pyruvic acid is the anionic form of an α-ketocarboxylic acid and, like other compounds of this general type, rapidly undergoes nonenzymatic decarboxylation in the presence of H_2O_2 to form acetate, carbon dioxide and water, according to this reaction: $CH_3COCOO^- + H_2O_2 \rightarrow CH_3COO^- + H_2O + CO_2$. In addition, pyruvate was shown recently to be capable of scavenging $OH^{\cdot}$ (12). Thus, in at least two ways, pyruvate is capable of limiting the cytopathic effects of various partially reduced forms of oxygen. Indeed, pyruvate, which normally is present in cells at millimolar concentrations, almost certainly serves as a crucial endog-

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enous scavenger of ROS (13). Moreover, administration of exogenous pyruvate has been shown to be protective in numerous *in vitro* and *in vivo* models of oxidant-mediated cellular or organ system injury (14–21).

Unfortunately, the usefulness of pyruvate as a therapeutic agent is abrogated by its very poor stability in solution (22, 23). Aqueous solutions of pyruvate spontaneously undergo an aldol-like condensation reaction to form 2-hydroxy-2-methyl-4-ketoglutarate, also known as parapyruvate (22, 24, 25). This compound can undergo spontaneous cyclization and dehydration to form the enolic lactone form or nonenzymatic reduction to form 2,4-dihydroxy-2-methylglutarate, which has been shown to be a mitochondrial poison (25). In an effort to take advantage of the ability of pyruvate to scavenge ROS while avoiding the problems associated with the instability of pyruvate in solution, we sought to determine whether a simple derivative, ethyl pyruvate, would be protective in an animal model of ROS-mediated tissue injury, namely mesenteric I/R in rats.

MATERIALS AND METHODS

The research protocol complied with the regulations regarding animal care as published by the National Institutes of Health and was approved by the Institutional Animal Use and Care Committee of the Beth Israel Deaconess Medical Center. All chemicals were purchased from Sigma Chemical (St. Louis, MO). Male Sprague-Dawley rats weighing 250–350 g (Taconic Laboratory, Germantown, NY) were used in this study. The animals were maintained at the Beth Israel Deaconess Medical Center Animal Research Center with a 12-hr light-dark cycle and free access to standard laboratory chow and water. Animals were not fasted before surgery.

Mesenteric I/R model. Studies of the effects of pyruvate and ethyl pyruvate solutions on gut mucosal injury secondary to mesenteric I/R were carried out by using methods similar to those previously described by Wattanasirichaigoon et al. (26, 27). Briefly, rats were anesthetized with intraperitoneal injections of sodium pentobarbital (30 mg/kg) and ketamine (20 mg/kg) and were maintained under anesthesia with intermittent intramuscular injections of ketamine (20 mg/kg) as needed. The animals were kept on a temperature-controlled surgical board (37°C). A tracheostomy was performed by using a short segment of polyethylene-120 tubing to keep the airway patent. The animals were allowed to breathe spontaneously. The right carotid and left jugular vein were cannulated with polyethylene-50 tubing. Mean arterial pressure was monitored by using a Transpac II transducer (Sorenson; Abbot Laboratories, North Chicago, IL) driving an amplifier/monitor with digital readout (7342A; Hewlett Packard, Santa Clara, CA). Heart rate was determined form the arterial pressure tracing. A syringe pump (Instech Laboratories, Plymouth Meeting, PA) allowed continuous intravenous fluid administration via the left jugular vein. Via a midline laparotomy, the superior mesenteric artery was dissected away from the surrounding connective tissue for a distance 1 cm from the aortic origin. Collateral circulation was isolated and ligated with 2.0 silk ties. After a 30-min stabilization period, the superior mesenteric artery was occluded with an atraumatic clip for 60 mins followed by 60 mins of reperfusion.

Experimental Protocol. Controls (n = 9) received intravenous lactated Ringer's solution (LR) containing 130 mM Na^+, 4 mM K^+, 2.7 mM Ca^{2+}, 109 mM Cl^-, and 28 mM lactate according this dosing schedule: 1.5 mL/kg bolus before ischemia, 3.0 mL/kg bolus before resuscitation, and 1.5 $mL \cdot kg^{-1} \cdot hr^{-1}$ by continuous infusion. We used LR as supplied by a commercial vendor, and pH was not adjusted. The experimental groups each received similar volumes of either pyruvate (n = 6) or ethyl pyruvate (n = 9) solution. The Ringer's pyruvate solution contained 130 mM Na^+, 4 mM K^+, 2.7 mM Ca^{2+}, 109 mM Cl^-, and 28 mM pyruvate (pH 7.0). The Ringer's ethyl pyruvate solution contained 130 mM Na^+, 4 mM K^+, 2.7 mM Ca^{2+}, 130 mM Cl^-, and 28 mM ethyl pyruvate (pH 7.0). To obtain tissues for assessing mucosal permeability and histology, five 10-cm long segments of small intestine were excised (taking care to ensure meticulous hemostasis) at the following time points: baseline (i.e., just before the induction of mesenteric ischemia), after 30 and 60 mins of ischemia (I30 and I60, respectively) and after 30 and 60 mins of reperfusion (R30 and R60, respectively). From each segment of small intestine, 8 cm was used to assess permeability, and the remaining 2 cm from each sample was fixed in 10% formaldehyde solution for histologic examination.

Measurement of Intestinal Mucosal Permeability. Intestinal mucosal permeability to the fluorescent tracer, fluorescein isothiocyanate dextran with a molecular weight of 4000 Da (FD4), was determined by using an everted gut sac method, as previously described by Wattanasirichaigoon et al. (27). Everted gut sacs were prepared in ice-cold modified Krebs-Henseleit bicarbonate buffer (KHBB, pH 7.4). One end of the gut segment was ligated with a 4.0 silk. The segment then was everted onto a thin plastic rod, and the resulting everted gut sac was secured with a 4.0 silk tied to the grooved tip of a 5-mL plastic syringe containing KHBB. The everted gut sac was distended gently by injecting 1.5 mL of KHBB. The everted gut sac then was suspended in a 100-mL beaker containing 80 mL of KHBB with added FD4 (20 μg/mL). The solution in the beaker was temperature jacketed at 37°C and was bubbled continuously with a gas mixture containing 95% O_2 and 5% CO_2.

A 1.0-mL sample was taken from the beaker before adding the everted gut sac to determine the initial external (i.e., mucosal surface) FD4 concentration. The everted gut sacs were incubated for 30 mins in the KHBB solution containing FD4. The length and volume of the gut sacs then were measured. The fluid on the serosal side was aspirated to determine FD4 concentration. The serosal and mucosal samples were centrifuged for 10 mins at 1000 × *g* and 4°C. We diluted 300 μg of the supernatant with phosphate-buffered saline (3.0 mL) and measured fluorescence by using a Perkin-Elmer LS-50 fluorescence spectrophotometer (Palo Alto, CA) at an excitation wavelength of 492 (slit width = 10 nm) and an emission wavelength of 515 nm (slit width = 10 nm). Permeability was expressed as the mucosal-to-serosal clearance of FD4 calculated using the following equations:

$$M = ([FD4]_{ser}) \times 1.5 \quad [1]$$

$$F = M/30 \text{ mins} \quad [2]$$

$$C = (F/[FD4]_{muc})/A \quad [3]$$

where M is the mass (in ng) of FD4 in the gut sac at the end of the 30-min incubation period; $[FD4]_{ser}$ is the FD4 concentration in the serosal fluid aspirated from the sac at the end of the 30-min incubation period, F is the flux of FD4 (in ng/min) across the mucosa, $[FD4]_{muc}$ is the FD4 concentration measured in the beaker at the beginning of the 30-min incubation period, A is the calculated area (in cm^2) of the mucosal surface, and C is the clearance of FD4 (in $nL \cdot min^{-1} \cdot cm^{-2}$) across the mucosa.

Histopathology. Fixed specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histologic sections were randomized, coded, and blindly interpreted (by CAS) at 100× and 400× magnification. (Note: CAS is listed as an "inventor" on a patent application for Ringer's ethyl pyruvate solution that has been filed with the U.S. Patent Office.) Mucosal lesions were graded on a scale of 0 to 5 by using criteria established by Chiu et al. (Table 1) (28). Polymorphonuclear cell infiltration also was graded by using the scale shown in Table 2. Villous height and mucosal thickness were determined by using a micrometer scale on the eyepiece lens. Ten individual readings were obtained from each slide, and mean measurements were reported.

Statistical Methods. Results are expressed as mean ± SEM. Significance of differences were determined by using analysis of variance and Fisher's least significant difference test. Differences were considered significant for $p < .05$.

ESULTS

In rats treated with LR or Ringer's pyruate solution, there was a significant decrease in mean arterial pressure relative to aseline after mesenteric reperfusion (Fig.). This reperfusion-induced decrease in ean arterial pressure, however, was attenated in rats treated with Ringer's ethyl yruvate solution, and at one time point 30), mean arterial pressure was signifiantly greater in the ethyl pyruvate group ompared with the control group. HR did ot differ significantly among groups (data ot shown). In controls infused with LR, ucosal permeability to FD4 increased at 0 by approximately six-fold and remained significantly greater than the baseline value at all subsequent time points (Fig. 2). Infusion of Ringer's pyruvate solution or Ringer's ethyl pyruvate solution tended to ameliorate the increase in permeability at the I30 and I60 time points, but the changes were not statistically significantly. However, treatment with either of the experimental solutions significantly ameliorated mucosal hyperpermeability during the reperfusion phase of the study.

Quantitative evaluation of hematoxylin and eosin-stained histologic sections showing villous height and mucosal thickness were significantly greater during both the ischemia and reperfusion phases in rats treated with ethyl pyruvate solution compared with controls treated with LR (Table 3). Mucosal injury scores at R60 were significantly lower in rats treated with ethyl pyruvate solution than in rats treated with LR. Infiltration of the mucosa by polymorphonuclear cells did not differ significantly among groups but was increased in all groups by the I/R protocol.

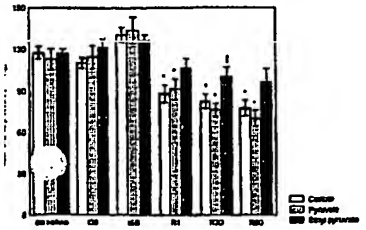
Figures 3–5 depict mucosal histology from representative sections. Normal mucosal histology is shown in Figure 3. Note in Figures 4 and 5 that there was virtually complete destruction of the mucosa when rats were treated with LR. Treatment with a Ringer's-type solution containing pyruvate partially preserved mucosal architecture. However, treatment with Ringer's-type solution containing ethyl pyruvate provided almost complete protection against mucosal injury.

able 1. Mucosal injury score as described by hiu et al. (28)

rade 1	Subepithelial space formation
rade 2	Moderate epithelial lifting confined to the tip of the villi
rade 3	Extensive epithelial lifting, few tips denuded
rade 4	Denuded villi, dilated exposed capillaries, increased cellularity in the lamina propria
rade 5	Hemorrhagic ulceration

able 2. Polymorphonuclear cell (PMN) infiltraon indexing score

	No PMN observed
	1–5 PMN per villus
	6–15 PMN per villus
	>15 PMN per villus and/or scattered venules filled with PMN adhering to endothelial walls
	Most venules (>80%) filled with PMN



igure 1. Effect of treatment with Ringer's pyruate or Ringer's ethyl pyruvate solution on mean rterial pressure in rats subjected to mesenteric schemia and reperfusion. The time points deicted along the abscissa are baseline (before the nset of ischemia), I30 and I60 (after 30 and 60 ins of ischemia, respectively), and R1, R30, and 60 (after 1, 30, and 60 mins of reperfusion, espectively). *$p < .05$ vs. the baseline value in he same group; †$p < .05$ vs. the time-matched alue in the control group (treated with lactated inger's solution).

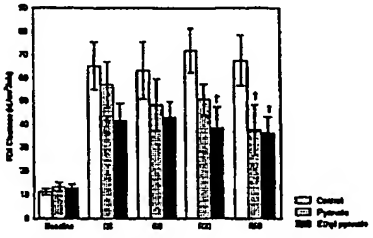


Figure 2. Effect of treatment with Ringer's pyruvate or Ringer's ethyl pyruvate solution on intestinal mucosal permeability to fluorescein isothiocyanate dextran with a molecular weight of 4000 Da (*FD4*) in rats subjected to mesenteric ischemia and reperfusion. The time points depicted along the abscissa are baseline (before the onset of ischemia), I30 and I60 (after 30 and 60 mins of ischemia, respectively), and R30 and R60 (after 30 and 60 mins of reperfusion, respectively). Clearance of FD4 increased significantly in all three groups studied after the onset of ischemia and remained significantly elevated relative to baseline through the final measurements at R60. †$p < .05$ vs. the time-matched value in the control group (treated with lactated Ringer's solution).

DISCUSSION

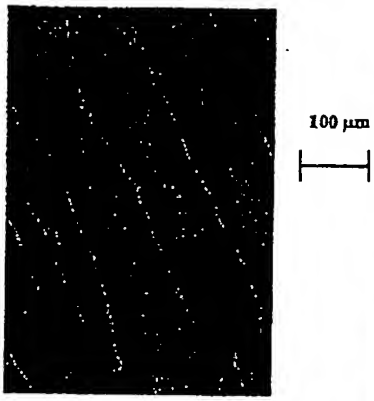
In the present study, we have confirmed previously published studies showing that treatment of experimental animals with a solution containing pyruvate can ameliorate tissue damage and organ dysfunction induced by I/R. In particular, we have confirmed and extended the findings obtained by Cicalese et al. (16), who also used a rat model of mesenteric I/R and documented that treatment with pyruvate decreased mucosal injury. Whereas Cicalese et al. focused on protection from histologic damage, we also assessed the effects on a functional parameter (mucosal permeability) of treating rats with pyruvate in a model of mesenteric I/R. Furthermore, Cicalese et al. (16) instilled their test solutions con-

Table 3. Histologic evaluation of hematoxylin and eosin-stained sections of intestinal mucosa at baseline, 60 min of ischemia (I60), and 60 min of reperfusion (R60)

Group	Timepoint	VH (μM)	MT (μM)	MIS	PMN
RL	Baseline	470 ± 30	553 ± 34	0.7 ± 0.3	0.8 ± 0.1
	I60	244 ± 20[a]	298 ± 32[a]	3.8 ± 0.3[a]	1.5 ± 0.2[a]
	R60	130 ± 25[a]	141 ± 22[a]	4.1 ± 0.4[a]	1.5 ± 0.2[a]
Pyruvate	Baseline	461 ± 25	524 ± 28	0.7 ± 0.4	0.7 ± 0.2
	I60	290 ± 30[a]	372 ± 36[a]	2.9 ± 0.5[a]	1.2 ± 0.1[a]
	R60	201 ± 44[a]	266 ± 50[a]	3.5 ± 0.6[a]	1.6 ± 0.2[a]
Ethyl pyruvate	Baseline	486 ± 12	583 ± 8	0.7 ± 0.2	0.7 ± 0.1
	I60	381 ± 24[a,b]	466 ± 25[a,b]	2.8 ± 0.4[a]	1.1 ± 0.1
	R60	296 ± 26[a,b]	352 ± 34[a,b]	2.4 ± 0.6[a,b]	1.3 ± 0.2[a]

LR, lactated Ringer's solution; VH, villus height; MT, mucosal thickness; MIS, mucosal injury score; PMN, neutrophilic infiltration score.

[a]$p < .05$ vs. the baseline value in the same group; [b]$p < .05$ vs. time-matched value in the LR control group.



Baseline

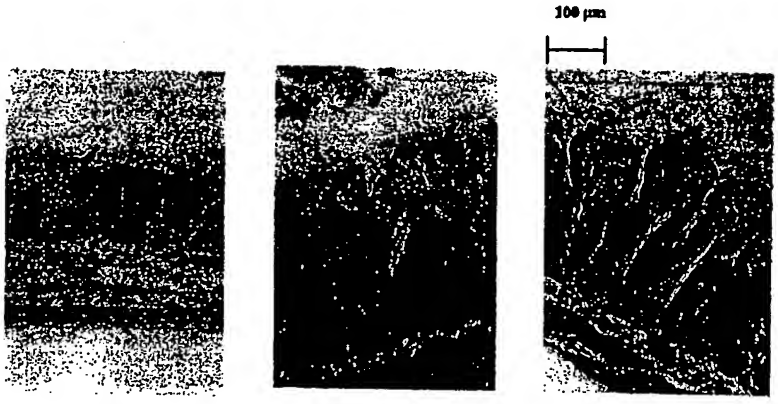
Figure 3. Photomicrograph of hematoxylin and eosin-stained section of normal rat small intestine.

I60: LR I60: Pyruvate I60: Ethyl pyruvate

Figure 4. Photomicrographs of hematoxylin and eosin-stained sections of rat small intestine after 60 mins of mesenteric ischemia (*I60*) and treatment with lactated Ringer's solution (*LR*), Ringer's pyruvate solution, or Ringer's ethyl pyruvate solution. Note that the mucosa is virtually completely destroyed in the specimen from a rat treated with LR, whereas the mucosal architecture is well preserved in the specimen from a rat treated with Ringer's ethyl pyruvate solution.

taining either sodium pyruvate or placebo (polyglucose) directly into the lumen of the gut before the onset of ischemia. In contrast, we administered the test solutions (LR, Ringer's pyruvate solution, or Ringer's ethyl pyruvate solution) intravenously, that is, in a way that might be directly applicable to the care of critically ill patients. Finally, Cicalese et al. administered pyruvate as a solution of the sodium salt of the α-ketocarboxylic acid. But, as noted earlier, pyruvate decomposes rapidly in aqueous solutions to a variety of products, including a toxic one (25) and, therefore, is not a candidate for development as a drug. Ethyl pyruvate, in contrast, seems to have attributes that make it a reasonable choice for further development as a therapeutic agent. To our knowledge, the effects of treating animals with a solution of ethyl pyruvate have not been reported before, except by us in abstract form (29). The only other published study evaluating the efficacy of ethyl pyruvate was a report by Varma et al. (30), who showed that ethyl pyruvate decreases oxidative damage to cultured lens cells.

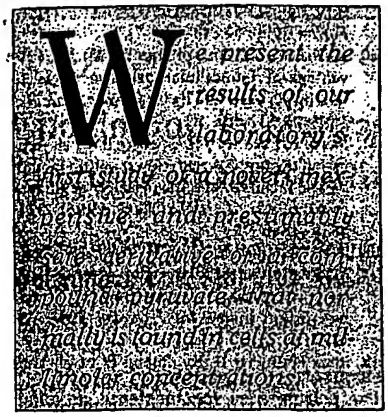
Ethyl pyruvate might offer several advantages with respect to pyruvate. For example, basic principles of organic chemistry suggest that ethyl pyruvate should be much more stable in solution than the parent α-ketoacid. Indeed, preliminary studies performed by one of the authors suggests that this prediction holds true (AMA, unpublished data, 2000). In addition, ethyl pyruvate, being considerably more lipophilic than pyruvate anion, should enter cells much more readily than the parent compound. Studies that used a related compound, methyl pyruvate, support this view (31). Additional studies that use cultured enterocytes are underway in our laboratory to specifically investigate the relative efficacy of pyruvate and ethyl pyruvate (as well as other compounds) as agents to protect cells against redox stress.



R60: LR R60: Pyruvate R60: Ethyl pyruvate

Figure 5. Photomicrographs of hematoxylin and eosin-stained sections of rat small intestine after 60 mins of mesenteric ischemia followed by 60 mins of reperfusion (*R60*). Rats were treated with lactated Ringer's solution (*LR*), Ringer's pyruvate solution, or Ringer's ethyl pyruvate solution. Note that the mucosa is completely destroyed in the specimen from a rat treated with LR, whereas the mucosal architecture is well preserved in the specimen from a rat treated with Ringer's ethyl pyruvate solution.

Despite the potential advantages of ethyl pyruvate as a pyruvate "pro-drug," before our studies there were no previous reports of this compound being useful as a component of a resuscitation fluid. One reason for the paucity of prior work with ethyl pyruvate may relate to its poor solubility in pure water (0.25% w/v; 22 mM). However, we discovered that the use of a balanced, calcium-containing salt solu-



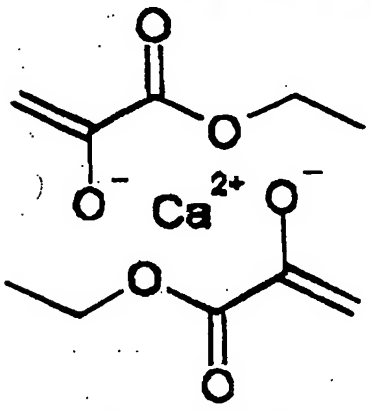


Figure 6. Proposed mechanism for stabilization of the enolate form of ethyl pyruvate by ionized calcium. Stabilization of the enolate form of the ethyl pyruvate by calcium ion markedly increases the solubility of the ester in an aqueous solvent.

tion (analogous to LR) markedly increases the solubility of ethyl pyruvate to 1.5% (w/v) or 130 mM. Based on preliminary nuclear magnetic resonance studies performed by one of the authors, the basis for the increased solubility of ethyl pyruvate in a Ringer's-type solution is stabilization of the enolate form of ethyl pyruvate by Ca^{2+}, as depicted in Figure 6 (AMA, unpublished data, 2000).

Although we presume that scavenging of ROS is the major reason for the beneficial effects of ethyl pyruvate solution observed in the present study, it also seems plausible that by acting as a pyruvate "pro-drug," ethyl pyruvate helps support anaerobic metabolism during hypoxia by driving the lactate dehydrogenase reaction toward lactate by a mass action effect and thereby fostering regeneration of oxidized nicotinamide adenine dinucleotide for use in the glyceraldehyde-3-phosphate dehydrogenase step of glycolysis. In this regard, it is noteworthy that the protective effects of pyruvate in a model of cardiac I/R-induced injury were attributed in a recent study to a decrease in the ratio of cytosolic reduced nicotinamide adenine dinucleotide to oxidized nicotinamide adenine dinucleotide (32). Additional studies that use a variety of cogeners of pyruvate and ethyl pyruvate will be necessary to clarify the relative contributions of the antioxidant and metabolic bases for the tissue protection observed in our experiments.

Our study has several limitations. First, we did not obtain direct biochemical evidence that treatment of rats with Ringer's ethyl pyruvate solution decreased oxidative damage (e.g., lipid peroxidation) initiated by mesenteric I/R. We are currently carrying out studies in a rat model of hemorrhagic shock and reperfusion in an effort to obtain such biochemical data.

Second, although we assessed systemic blood pressure, we did not directly measure gut mucosal perfusion. Accordingly, we cannot exclude the possibility that some (or even all) of the protective effects of Ringer's ethyl pyruvate were related to better perfusion of the intestine after restoration of mesenteric blood flow. However, based on prior studies from our laboratory that used the same model of mesenteric I/R (26), we are confident that intestinal microvascular blood flow is almost nil (<4% of baseline) during the ischemic phase of the protocol. Accordingly, it seems highly improbable that maintenance of perfusion when the mesenteric vasculature was cross-clamped was responsible for the protection observed in the present study with Ringer's ethyl pyruvate.

Third, LR might not be the ideal control resuscitation fluid for studies like the one described herein. Compared with other resuscitation fluids, including whole blood, hypertonic saline solution, and plasma, LR causes greater activation of polymorphonuclear neutrophils (33) and apoptosis of cells in the liver, lung, and mucosa and muscularis propria of the gut (34, 35). By the same token, however, LR is preferable from a physiologic standpoint to at least one other commonly used intravenous resuscitation fluid, normal saline (36, 37). Thus, because LR is still commonly used in hospitals to resuscitate patients with a variety of shock-like conditions and because it may be preferable to at least [redacted] another commonly used intravenous [redacted]loid solution (normal saline), we believe that the control solution for the present studies was a reasonable choice.

In summary, we present the results of our laboratory's first study of a novel, inexpensive, and presumably safe derivative of a compound, pyruvate, that normally is found in cells at millimolar concentrations. The results presented here support the view that this derivative, ethyl pyruvate, when formulated in a calcium-containing balanced salt solution, warrants additional evaluation as a therapeutic agent for the treatment of a variety of conditions associated with tissue ischemia and/or oxidative stress.

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